## ABSTRACT

Isoelectric focusing systems are used to analyze ampholytic analytes in a sample. These systems use an electrophoretically generated pH gradient to separate components according to their isoelectric points. This invention overcomes two shortcomings associated with these systems. First, the invention enables the detection of ampholytic analytes whose original concentration in a sample is so low that their concentration after focusing is below their respective detection limit. Auxiliary agents are added to the sample and auxiliary compartments are connected to the separation compartment to increase the final concentration of the focused ampholytic analytes in the separation compartment above their respective detection limit. The second limitation the invention overcomes is the detrimental effects of salt in a sample. Salt alters the pH gradient developed in the separation compartment during focusing compared to the pH gradient obtained for a salt-free sample, thus skewing the electropherogram obtained in the isoelectric focusing separation. This invention eliminates the problems caused by salt-induced shift of the pH gradient by accumulating, during isoelectric focusing, components of salt in the sample and the added auxiliary agents in an auxiliary compartment connected to the separation compartment. By adjusting the amount of auxiliary agent so that at the end of the focusing step no salt or auxiliary agent is located in the separation compartment, one can maintain the correct shape of the pH gradient in the separation compartment, increase the concentration of the focused ampholytic analyte above its respective detection limit and avoid the unwanted effects of salt in the sample.